CHARACTERISTICS AND ROLE OF THE MITE, *PHYLLOCOPTES FRUCTIPHILUS* (ACARI: ERIOPHYIDAE) IN THE ETIOLOGY OF ROSE ROSETTE[1,2]

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ABSTRACT: Large numbers of *Phyllocoptes fructiphilus* (Acari: Eriophyidae) were found on naturalized multiflora roses, *Rosa multiflora,* in central Missouri. Although the greater numbers occurred on plants with symptoms of rose rosette, *P. fructiphilus* neither induced symptoms of rose rosette with salivary compounds nor transmitted the infectious agent. The role of this mite in the etiology of rose rosette, therefore, remains unclear. SEM micrographs indicate the value of scanning electron microscopy as a tool in the study of mite taxonomy.

Rose rosette was first described on wild roses in the northwestern United States (Thomas and Scott, 1953) and southwestern Canada (Conners, 1941, 1942). Recently, it has been found on wild and ornamental roses in central United States (Crowe, 1983; Doudrick and Millikan, 1983). Typical symptoms includes witches' brooming, altered coloration and development of leaflets (Keifer et al., 1982), and a phylloid condition of the flowers (Doudrick, 1984). Symptoms of rose rosette, however, vary among species and between cultivars of garden roses. Although the etiology of rose rosette and the identity of the infectious agent have not been established, the infectious agent has been transmitted by the eriophyid mite, *Phyllocoptes fructiphilus* Keifer (Allington et al., 1968). Root grafting, however, may be involved in the rapid spread within an area after initial infection. It has been suggested by Keifer (pers. comm.) that the symptoms of rose rosette may result from a toxicogenic substance associated with mite feeding. Recently, Gergerich and Kim (1983) described virus-like particles (VLP) which were associated with rose rosette and suggested that VLP might be the causal agent.

The purpose of this study was to determine the role of eriophyid mites in the transmission and etiology of rose rosette, and to characterize morpholog-

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ical features of the mite by means of scanning electron micrographs.

MATERIALS AND METHODS

A thornless selection of multiflora rose, *Rosa multiflora* Thunberg, Clone 1, was used in the study. The possible role of *P. fructiphilus* in rose rosette was examined by grafting mite-free internodal shields from a multiflora rose with symptoms of rose rosette to 10 healthy Clone 1 plants. These plants were maintained in a separate greenhouse, treated with a rotation of miticides, and periodically examined for mites. At the end of 14 wks, three plants were critically examined for eriophyid mites.

The resident population of mites on several naturalized multiflora roses was evaluated in the late spring and early summer of 1983. Shoots of current season's growth were randomly collected from plants with and without symptoms of rose rosette and examined for the presence and identity of mites. Because these mites appeared to be a single eriophyid species, they were tentatively identified and representative specimens prepared for scanning electron microscopy (SEM). The mites were mounted on a foil-backed tape attached to copper stubs and fixed for 2 hrs in a small droplet of aqueous 1% osmium tetroxide placed on the surface of the tape. Fixed specimens were dehydrated by 10 min exposures in a graded series of ethanol (20, 40, 60, 80, 95, 100, 100, 100%), critical point dried (Anderson, 1951), and sputter coated with gold prior to examination in the JEOL-JSM-S1 SEM operated at 10 kV.

Several attempts were made to establish a colony of mites and transmit the rose rosette agent (RRA) by *P. fructiphilus.* In one experiment, ten mites were placed on the tips of each of ten healthy Clone 1 plants. These were maintained at 17°-20°C in an isolated dark room and exposed to fluorescent lighting with a 16:8 hr light:dark cycle. Five plants were covered with glass jars similar to growth chambers of del Rosario and Sill (1958), and five were left uncovered. Another experiment in a greenhouse involved the placing of eight adults, 1 nymph and 3 eggs of *P. fructiphilus* on the tips of each of 30 healthy Clone 1 plants. In a third experiment in August, 1983, 50 mites from a multiflora rose with symptoms of rose rosette were transferred to the tips of an inoculated Clone 1 with symptoms of rose rosette. Two weeks later ten adult mites were transferred to the tips of a healthy Clone 1, and 5 adults and 1 nymph were transferred to another healthy Clone 1. Unfortunately, before additional mites could be transferred from this source, this colonized multiflora died. All healthy Cone 1 plants used in the mite transfer studies were maintained and observed for periods of time ranging from 20 wks to 12 mos.

RESULTS

All 10 of the healthy Clone 1 plants which had been grafted with mite-free internodes from the rose rosette-infected plant became infected with rose rosette in 6 - 14 wks after inoculation. After the development of symptoms, no mites, shed skins, or eggs of *P. fructiphilus* were found by microscope examination of buds (2080) and tips (208) of 3 of these plants.

A great number of mites were found associated with the leaf axils and buds of naturalized multiflora roses, generally around the bud and beneath the bud scales. Mites were present in these locations on the shoots from apparently healthy plants, as well as those with symptoms of rose rosette. The average number of mites, however, was higher on the buds and tips from shoots of diseased plants (6.0/bud and 29.2/tip) than in the buds and tips from apparently healthy shoots (1.6/bud and 2.8/tip). Shoots from the diseased plants had a maximum of 367 mites whereas those from the apparently healthy plants had a maximum of 74. Occasionally, no mites were found on the shoots from either the healthy or diseased sources.

Based on SEM micrographs, the adult females are spindle shaped and 140 - 180 μm in length (Fig. 1). The dorsal shield is nearly triangular with the anterior lobe broadly pointed and projecting over the rostrum (Fig. 2). The shield pattern consists of a network of ridges with the medial ridge present only in the rear. A pair of tubercles project from just anterior of the rear margin of the dorsal shield and bear long setae which point upward and toward one another. The featherclaws are five-rayed and further subdivided into lateral branches (Fig. 3). The microtubercles are spaced close together and cover the entire abdomen (Fig. 4). These microtubercles are generally rounded or elliptical in shape with some on the sternites along the rearmost portion of the abdomen, tending to be more pointed. The female genital coverflap has 6 - 8 longitudinal ridges and appears to hinge actively (Fig. 5 and 6).

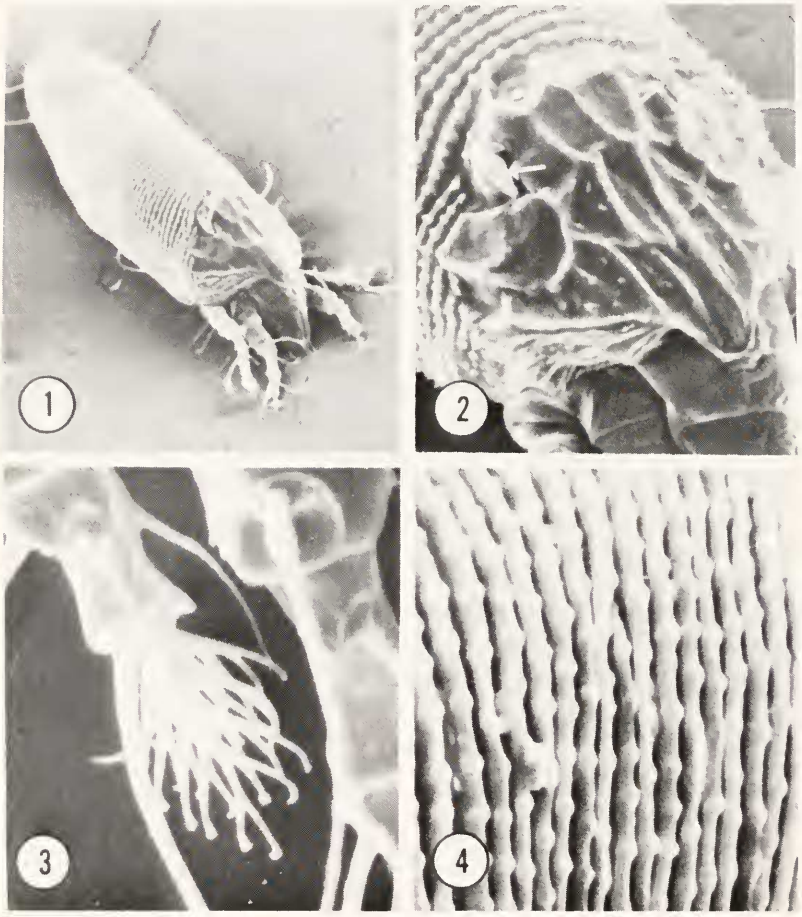
The adult males, although smaller, closely resemble the females. The external genitalia of the males consist of a traverse opening (Fig. 7) with sensory pegs located just around the opening. These micrographs were examined by Dr. George Oldfield, Boyden Laboratory, University of California-Riverside, who confirmed the identity of the mites in this study as *P. fructiphilus.*

DISCUSSION

The multiflora rose population in central Missouri supports large numbers of eriophyid mites. These mites are found in and around the buds and tips of apparently healthy plants, as well as those with symptoms of rose

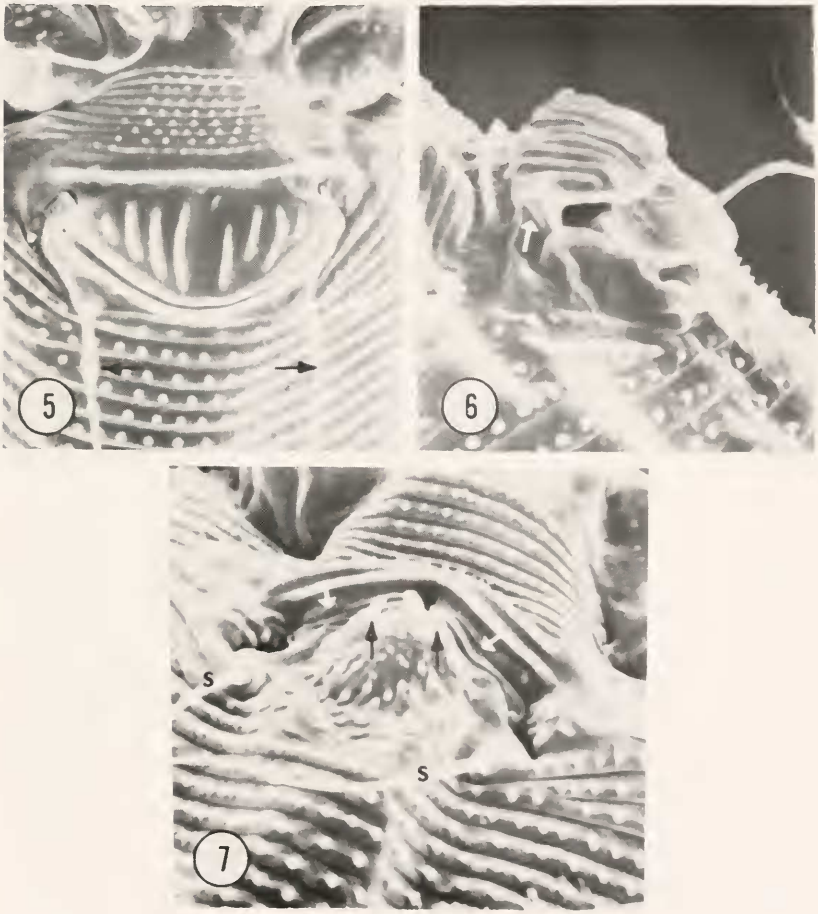
rosette. The diseased plants, however, support a much larger number of mites. These mites appear to be a single species and were identified as *P. fructiphilus.*

Our studies demonstrate the usefulness of SEM in mite taxonomy. SEM permits a more economical use of time with more precision than drawing. The use of SEM also provides a means of rapid identification of mites without the problems associated with interstate shipments of mites.



Figs. 1-4. Scanning electron micrographs of *Phyllocoptes fructiphilus.* 1. An adult female (x360). 2. Dorsal shield of adult showing the network of ridges and tubercles (arrows) bearing slender setae (x1,200). 3. Five-rayed featherclaw (x4,500). 4. Dorsal portion of abdomen bearing round to elliptical microtubercles (x2,700).

Our efforts to transmit RRA by *P. fructiphilus* were unsuccessful, possibly due to the source of mites. In those tests where transmission was reported (Allington et al., 1968), *P. fructiphilus* was collected from infected *R. eglanteria* L., *R. eglanteria* hybrid, *R. suffulata* Greene, and *R. woodsii* Lindl. No transmission was obtained when mites from other rose species, including multiflora, were used. Our inability to establish colonies



Figs. 5-7. Scanning electron micrographs of *Phyllocoptes fructiphilus*. 5. Ventral view of female genitalia showing genital setae (arrow) and longitudinal ridges of the coverflap (x1,600). 6. Lateral view of female genitalia with anterior hinging (arrow) of the coverflap (x2,400). 7. External genitalia of male illustrating the transverse opening (white arrows), sensory pegs (black arrows) and setae (S) (x2,400).

of *P. fructiphilus* on healthy Clone 1 plants prevented more detailed studies on transmission. Similar results were reported by Allington et al., (1968), who found that either the mites failed to survive on multiflora or that the host became so infested with the two spotted spider mite, *Tetranychus urticae* Koch, that the plants had to be destroyed.

The role of *P. fructiphilus* in the rose rosette disease remains unclear. However, the transmission of RRA by mite-free internodal shields to healthy plants maintained in a mite-free environment indicates that a toxicogenic substance associated with mite feeding is not crucial to the development of symptoms of rose rosette. This same graft transmission supports the role of an identified virus-like organism as the etiologic agent of rose rosette. In spite of these findings and our inability to demonstrate transmission, the greater numbers of *P. fructiphilus* on roses with symptoms of rose rosette indicate that this disease represents an unusual mite: host association that merits further study.

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REFERENCES

Anderson, T.F. 1951. Techniques for preservation of three dimensional structure. Trans. New York Acad. Sci. 13: 130-134.

Allington, W.B., R. Staples, and G. Viehmeyer. 1968. Transmission of rose rosette by the eriophyid mite, *Phyllocoptes fructiphilus* J. Econ. Entomol. 61:1137-1140.

Conners, I.L. 1941. Twentieth Ann. Rep. Canadian Plant Dis. Survey, 1940: 102.

Conners, I.L. 1942. Twenty-first Ann. Rep. Canadian Plant Dis. Survey, 1941: 102.

Crowe, F.J. 1983. Witches' broom of rose: a new outbreak in several states. Plant Dis. 67: 544-546.

del Rosario, M.S. and W.H. Sill, Jr. 1958. A new method of rearing large colonies of an eriophyid mite, *Aceria tulipae* Keif., in pure culture from eggs or adults. J. Econ. Entomol. 55: 303-306.

Doudrick, R.L. 1984. Etiological studies of rose rosette. 101 p. Unpublished MS thesis, University of Missouri-Columbia library.

Dourdrick R.L. and D.F. Millikan. 1983. Rose rosette, a disease of multiflora and garden roses. Trans. Missouri Acad. Sci. 17 (Abstr): 191.

Gergerich, R.C. and K.S. Kim. 1983. Description of the causal agent of rose rosette. Arkansas Farm Res. May-June: 7.

Keifer, H.H., E.W. Baker, T. Kono, M. Delfinado, and W.E. Styer. 1982. An illustrated guide to plant abnormalities caused by the eriophyid mites in North America. U.S. Department of Agriculture, ARS. Agr. Handbook No. 573, pp. 164-165.

Thomas, H.E. and E.C. Scott. 1953 Rosette of rose. Phytopathology 43: 218-219.

EGG CAPSULE MORPHOLOGY OF *ANISOMORPHA BUPRESTOIDES* (PHASMATODEA: PSEUDOPHASMATIDAE)[1]

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ABSTRACT: Egg capsule morphology of the stick insect *Anisomorpha buprestoides* (Stoll) is observed with scanning electron microscopy. The length of the capsule is 2.61 mm and the width is 1.82 mm. The irregular capsule surface is covered throughout with smooth tubercles and scattered umbrella-like projections. These projections may represent a unique feature for this species.

The eggs of many stick insects remain unstudied at both the light microscopy and scanning electron microscopy (SEM) levels. Due to lower resolution capabilities, light microscope studies of stick insect capsules tend to present surface features less accurately and in less detail. With the use of SEM, however, the structure of the capsule can be observed closely and in great detail.

According to Viscuso and Longo (1983), morphological characteristics of the chorion should provide useful data for taxonomic and phylogenetic purposes, and this viewpoint is supported by recent SEM studies on eggs of stick insects (Godeke and Pijnacker, 1984; Mazzini *et al.*, 1984; Mazzini and Scali, 1977, 1980; Scali and Mazzini, 1977, 1982, 1983; Stark and Lentz, 1986). Using light microscopy, Clark (1976) studied the eggs of many stick insects, including *Anisomorpha buprestoides* (Stoll), a species common in Mississippi and other southeastern states. In this study we define the ultrastructure of the egg capsule of *A. burprestoides* at the SEM level and add information to Clark's (1976) light microscopy study.

MATERIALS AND METHODS

Eggs of the stick insect, *A. buprestoides,* were obtained from a caged female collected in Hinds County, Mississippi, during October, 1985. Eggs were placed in 70% ethanol and examined under a dissecting stereomicroscope. If debris was detected, the eggs were placed in distilled water and agitated in an ultrasonic cleaner for 30 sec. The eggs were dehydrated in acetone, air-dried, placed directly on specimen stubs with silver conducting paint, and coated with a 40 nm layer of gold using a Hummer II Sputter Coater set for 2 min at 10 mamp. The eggs were studied with an AMRay

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1000 scanning electron microscope with the stage tilted at a 45° angle and an accelerating voltage of 20 KeV. Micrographs (Figs. 1-12) are listed with original magnifications; terminology follows Clark (1976).

RESULTS

The overall appearance of the egg capsule of *A. buprestoides* is shown in Fig. 1. The eggs are black with a mean capsule length of 2.61 ± 0.1 mm (range = 2.4-2.7) and a mean width of 1.82 ± 0.04 mm (range = 1.77-2.0). The chorionic surface consists of many irregular ridges and valleys covered with smooth tubercles (Fig. 2) which vary in size and are interconnected by thin filaments (Fig. 3). Elevated, large, umbrella-like projections resembling liverwort antheridiophores (Fig. 4) are scattered in groups of two or more over the surface (Fig. 5). Each projection consists of a smooth stalk and an irregular, warty anterior surface. Openings or punctations appear to be present on the anterior portion of these projections (Fig. 6).

The elliptical operculum (op) is convex, consisting of a flat peripheral rim and a central area with the same surface characteristics as the capsule (Figs. 7, 8). Capitular structures are absent.

The micropylar plate (mp) is a concave structure 0.9 mm long and 0.4 mm wide, surrounded by an elevated rim on the mid-dorsal surface of the egg (Figs. 1, 9, 10). The mp surface is similar to that of the capsule, except the umbrella-like projections are absent (Figs. 10, 11). The median line (ml) extends 0.3 mm from the base of the micropylar plate to the posterior pole of the capsule (Figs. 1, 10). An irregular median tubercle (mt) lies just above the indistinct micropylar cup (mc) (Fig. 10). Micropylar orifices are absent.

The porous exochorion (ex) is less than 0.01 mm thick and contains numerous large spaces (Fig. 12). The endochorion (en) has a thickness of 0.2 mm and consists of a dense meshwork of closely packed fibers without interstitial spaces (Fig. 12).

DISCUSSION

The information presented here is the first report of egg capsule morphology of *A. burpestoides* using SEM. While confirming all the main structures described by Clark (1976) using light microscopy, more specific capsule characteristics were revealed with SEM. Clark's (1976) reference to a "mottled surface" actually consists of numerous tubercles with umbrella-like projections. These projections are scattered over the surface of the egg capsule and operculum but are absent on the micropylar plate. The umbrella-like projections are presently known to occur only on the egg of this species. Although their exact function is not known, they may serve

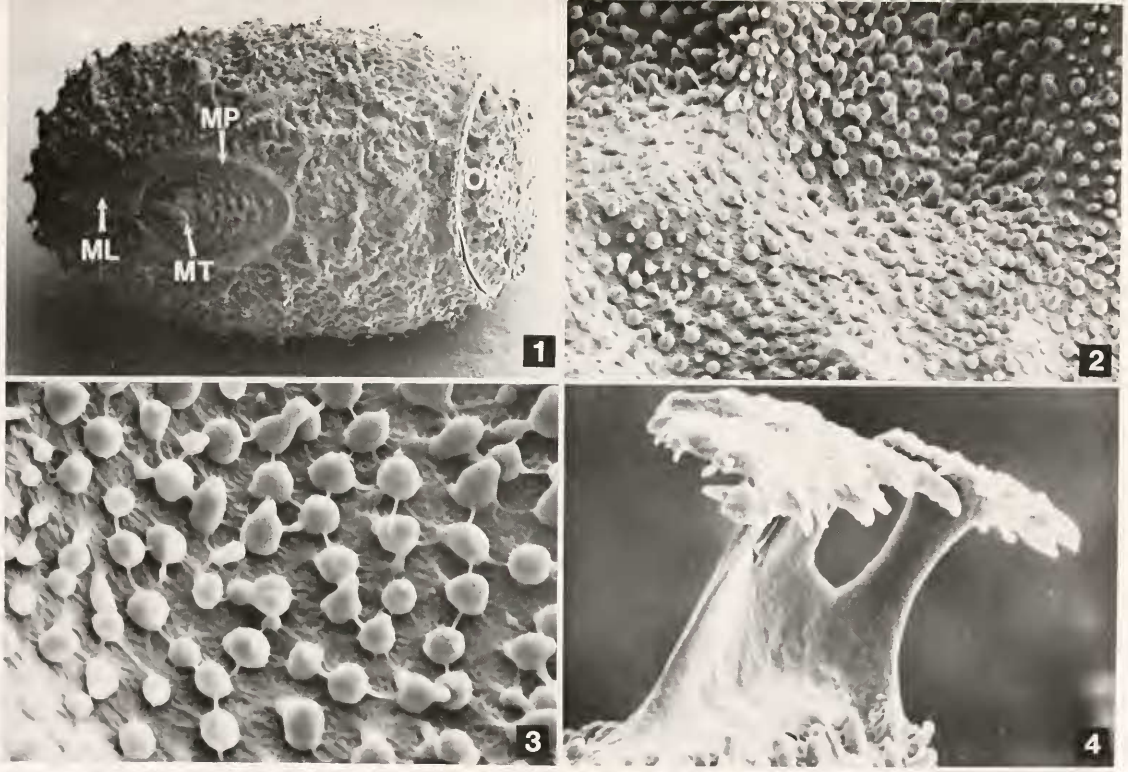

Fig. 1. *Anisomorpha buprestoides* egg capsule, dorsal aspect (32 X), ml = median line, mt = median tubercle, mp = micropylar plate, op = operculum (terminology follows Clark, 1976).
Fig. 2. Egg capsule surface, showing smooth tubercles (990 X).
Fig. 3. Egg capsule surface detail, showing connecting filaments between tubercles (1980 X).
Fig. 4. Umbrella-like projections on the egg capsule surface (1440 X).

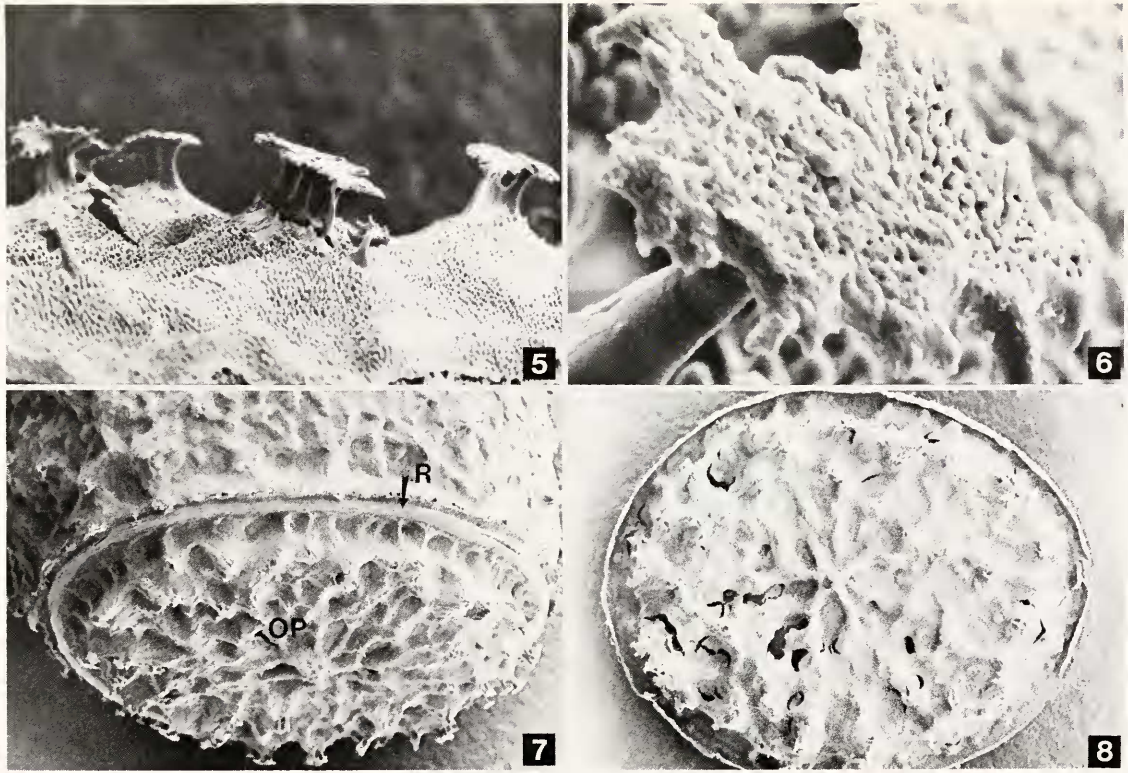

Fig. 5. Egg capsule surface, showing clustering of umbrella-like projections (308 X).
Fig. 6. Umbrella-like projections, showing irregular, warty anterior surface and smooth stalk (2420 X).
Fig. 7. Operculum showing its overall concavity and indentations adjacent to the rim (50 X). op = operculum, r = rim.
Fig. 8. Detail view of isolated operculum (60 X).